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C-Terminal Agrin Fragment – A New Fast Biomarker for Kidney Function in Renal Transplant Recipients

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Key Words
Kidney function · Glomerular filtration rate · Creatinine · C-terminal agrin fragment · Biomarker · Transplant · Delayed graft function

Abstract
Background: The C-terminal agrin fragment (CAF) is a cleavage product of agrin, the major proteoglycan of the glomerular basement membrane. This article studies if CAF could serve as a biomarker for renal function in renal transplant recipients. ***Material and Methods:*** We measured serum CAF and creatinine concentrations and calculated estimated glomerular filtration rate (eGFR) (MDRD) in 96 healthy individuals and in 110 end-stage renal disease patients undergoing kidney transplantation before and after transplantation. Correlation between CAF and creatinine concentrations/eGFR was calculated as within-patient (cWP) and between-patient correlations (cBP). Moreover, we evaluated the association of CAF with delayed graft function (DGF). The diagnostic value of CAF for early detection of DGF compared to creatinine was evaluated by receiver operating characteristics (ROC) analysis. ***Results:*** CAF concentrations strongly correlated with creatinine (r = 0.86 (cWP), r = 0.74 (cBP)) and eGFR (MDRD) (r = 0.86 (cWP), r = 0.77 (cBP)). Pre-transplant (pre-Tx) CAF concentrations were 19-fold higher than in healthy individuals (1,115.0 (258.4–3,990.0) vs. 56.6 (20.0–109.5) pM). After transplantation, CAF decreased significantly faster than creatinine (postoperative days 1–3 (POD 1–3): 562.8 (101.6–2,113.0) pM; creatinine: pre-Tx 6.9 (3.1–15.7), POD 1–3: 6.4 (1.7–12.7) mg/dl, p < 0.001). Stable concentrations were reached 1–3 months after transplantation for CAF and creatinine (CAF 145.1 (6.7–851.0) pM; creatinine 1.6 (0.7–8.0) mg/dl). CAF concentrations at POD 1–3 were significantly associated with DGF and outperformed creatinine in early detection of DGF (area under the curve (AUC) CAF 80.7% (95% CI 72.3–89.1%) vs. AUC creatinine 71.3% (95% CI 61.8–81.1%), p = 0.061). ***Conclusion:*** CAF is a promising new and fast biomarker for kidney function and may serve as a new tool for the early detection of DGF.

Introduction

Serum creatinine and urea are the most widely used biomarkers to monitor kidney function [1, 2]. Although they have been used over decades their application is lim-

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ited: they lack sensitivity and specificity, especially in acute kidney injury, and are influenced by multiple parameters such as muscle mass, liver function, and pharmacological substances [1, 2]. Thus, new biomarkers have been evaluated, such as cystatin C, human neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18 (IL-18) and kidney-injury molecule 1 (KIM-1) [3, 4]. But so far, only cystatin C measurements have in part been established for routine diagnostics.

Neurotrypsin, a serine protease, cleaves agrin, a major heparan sulfate proteoglycan, at two homologous sites, releasing a 22-kDa C-terminal fragment, called 'CAF' [5, 6, see also suppl. 2 in 7]. Among neuronal and other tissues, agrin is highly expressed in the kidney, where it substantially contributes to the formation of the glomerular basement membrane [8, 9]. Circulating CAF is detectable in human blood. We hypothesized that changes in kidney function may be associated with changes in CAF serum concentrations in humans. So far, CAF has never been explored as a novel marker of renal function.

Here we characterized CAF as a biomarker for kidney function. We evaluated serum CAF concentrations in healthy subjects that did not undergo any intervention and in 110 renal transplant recipients before and at various time points after transplantation to correlate CAF concentrations with creatinine and estimated glomerular filtration rate (eGFR). Additionally, we addressed whether early postoperative CAF concentrations could detect a delay of graft function (DGF) more accurate than creatinine in the short term after transplantation.

Table 1. Kidney allograft recipients' demographics

Parameter	Result
Age, years	51.2±13.5
Gender	110 (100)
Male	71 (64.5)
Female	39 (35.5)
Transplantation	110 (100)
Kidney	103 (93.6)
Kidney-pancreas	7 (6.4)
Kind of donation	110 (100)
Deceased donor	79 (71.8)
Living donor	31 (28.2)
Underlying renal disease	110 (100)
Diabetic nephropathy	23 (20.1)
Vascular nephropathy	7 (6.4)
Autosomal polycystic kidney disease	11 (10.0)
Immunogenic (IgA nephropathy, GwP, LE, MPGN)	34 (30.9)
Other (hereditary, intersitial disease, unknown)	35 (31.8)
Immunosuppression – calcineurin inhibitor	
Tacrolimus	67 (60.1)
Cyclosporine	23 (20.1)
Switch from cyclosporine to tacrolimus	16 (14.5)
Switch from tacrolimus to cyclosporine	4 (3.6)
Patients with delayed graft function	40 (36.4)
Samples obtained in total	746 (100)
Before transplantation	110 (14.7)
After transplantation	
1–3 days	127 (17.0)
4–10 days	131 (17.6)
11–30 days	98 (13.1)
30–89 days	123 (16.5)
90–179 days	88 (11.8)
6–12 months	48 (6.4)
>12 months	21 (2.8)

Values are mean ± SD or n (%). GwP = Granulomatosis with polyangiitis; LE = lupus erythematosus; MPGN = membranoproliferative glomerulonephritis.

Patients and Methods

Study Population

The study was approved by the local Ethics Committee of Klinikum rechts der Isar, Technische Universität, Munich, Germany. All patients enrolled in this study gave their consent. The total study population consisted of 206 individuals and was based on an observational study concept. The data were analyzed retrospectively. Two different groups of patients were included: 96 healthy volunteers who did not undergo any intervention and 110 patients suffering from chronic kidney disease undergoing kidney transplantation.

Kidney Transplant Recipients

Patients underwent kidney (living as well as postmortal donors) or combined kidney-pancreas transplantation in the time from 2007 to 2011 at Klinikum rechts der Isar. No specific inclusion or exclusion criteria had to be met. All patients received an initial triple immunosuppression consisting of a calcineurin inhibitor (71 TAC, 39 CyA), mycophenolic acid and corticosteroids (table 1). In the follow-up period, 16 patients were switched from CyA to TAC, whereas 4 patients were switched from TAC to CyA. Blood samples were obtained on the day of surgery before transplantation and several times up to a median of 128 days after transplantation (range 6–1,757). In total, 746 samples were obtained. The time points when blood was drawn did not follow a strict protocol, but blood samples could be obtained from every patient before and at least once in the early postoperative period (postoperative days (POD) 1–3). Blood samples were categorized into certain time periods after transplantation (table 1). If more than one sample from 1 patient was obtained during a specific study period, all samples were analyzed, the mean CAF level calculated and used for statistical analysis.

Serum samples were analyzed for CAF/creatinine concentrations and eGFR (MDRD) [10] was calculated. To analyze patient

characteristics that might influence CAF concentrations, age, gender, weight, body mass index (BMI), underlying disease (grouped as indicated in table 1), hemoglobin, sodium and total protein concentrations, inflammatory status (total leukocyte count (TLC) and C-reactive protein (CRP)) and liver damage parameters (γ-glutamyltransferase (GGT) and glutamate pyruvate transaminase (GPT)) were analyzed. At last we assessed the incidence of DGF, defined as the need for at least one dialysis treatment within the first week after transplantation [11]. The need for dialysis treatment was evaluated by the treating physician and did not follow a strict protocol.

Healthy Volunteers

The control group consisted of 96 healthy volunteers. Blood samples were drawn once in the morning hours to measure serum CAF and creatinine concentrations.

Blood Sample Measurement of CAF and Creatinine Concentrations

All blood samples were evaluated for CAF concentrations using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (NTCAF Elisa Kit; Neurotune, Schlieren, Switzerland [12], see a detailed description there). In brief, 50 μl of blood sample was mixed with 50 μl of incubation buffer in Deepwell protein LoBind plates. Then 100 μl of 400 nM CAF calibrator protein solution was mixed with 900 μl dilution buffer in order to create a calibrator dilution series on the same plate. The plate was then sealed and incubated in a water bath at 56 ± 1°C for 30 ± 1 min. After centrifugation for 5 min at 3,000 *g* at room temperature, 10 μl of sample and dilution series was transferred to a pre-coated microtiter plate, which had already been prepared with 90 μl of dilution buffer in each well. The ELISA plate was incubated for 16 h at room temperature. The plate was washed 3 times and 100 μl of CAF detector antibody solution was added to each well and incubated for 30 min at room temperature. The same step was repeated with SA-poly-HRP solution, followed by TMB solution for color development. The results were read out on a plate reader at 450 nm. Data were analyzed using an Excel file supplied by the company. CAF values are expressed as picomolar (pM), which corresponds to a concentration of 20 pg/ml.

Serum creatinine concentrations were quantified using a well-established photometric measurement (Jaffe method, normal range 0.7–1.3 mg/dl in males and 0.5–1.1 mg/dl in females). Measurements were conducted at the Institute of Clinical Chemistry at Klinikum rechts der Isar, Central Laboratory Service.

Statistics

For statistical analysis, IBM SPSS 20 and R 2.15.1 (R Foundation for Statistical Computing, Vienna, Austria) were used. The Kolmogorov-Smirnov test was performed to evaluate the normality of data distribution. Continuous data are expressed as mean ± standard deviation or median (range) whenever appropriate. Categorical variables are reported in absolute numbers and percentages. To assess correlation between CAF and creatinine concentrations/eGFR in renal transplant recipients, a within-patient correlation (cWP, in case of at least three samples obtained in 1 patient) as well as a between-patient correlation (cBP) was calculated [13, 14]. We tried to answer two questions: we used the cWP to analyze whether changes in CAF concentrations in 1 patient were associated with changes of creatinine concentrations in the same patient in order to remove variability between patients. To determine whether patients with high average CAF concentrations also tend to have high average concentrations of creatinine, we used the cBP. Because the relationship between CAF and creatinine as well as eGFR followed a power function, which can be linearized by a log transformation, we calculated correlation between logarithmic CAF and logarithmic creatinine/eGFR values. To test if there is a kinetic difference in the decrease of postoperative CAF and creatinine concentrations compared to pre-transplant (pre-Tx) and to compare CAF and creatinine concentrations between different time periods, the Wilcoxon signed rank test was used. Pairwise correlations (using Spearman ρ correlation coefficients) were performed between pre-Tx CAF concentrations and the following covariables: age, weight, BMI, GPT, GGT, TLC, CRP, hemoglobin, sodium and total protein concentrations. To analyze differences in pre-Tx CAF concentrations with respect to gender as well as to evaluate if there is a difference of median CAF and creatinine concentrations on POD 1–3 between patients with and without DGF, we utilized the Mann-Whitney U test. The Kruskal-Wallis test was used to evaluate possible differences between serum CAF concentrations and the underlying disease. Additionally, a receiver operating characteristic (ROC) analysis was performed to evaluate if CAF is an appropriate marker for early detection of DGF on POD 1–3 with accurate sensitivity and specificity and it was then compared to creatinine. All analyses were done using a two-sided 0.05 level of significance and have not been adjusted for multiple testing.

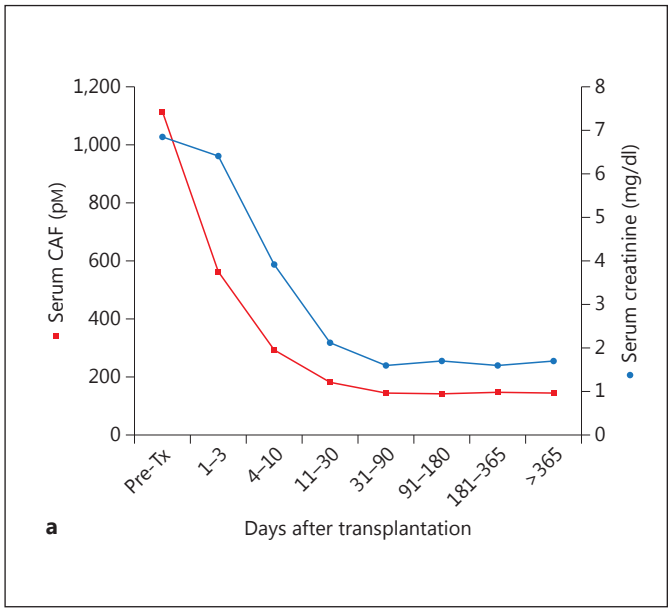
Results

Patients' Demographics

The mean age of kidney transplant recipients was 51.2 ± 13.5 years, 71 (64.5%) patients were male (table 1). 103 patients received single kidney transplantation, in 79 patients a deceased donor organ was transplanted. The mean age of healthy volunteers was 47.7 ± 16.0 years, 34 (36.1%) were males. In healthy volunteers, CAF concentrations were 56.0 ± 18.5 pM, creatinine concentrations 0.78 ± 0.13 mg/dl, and eGFR was 95 ± 17 ml/min.

Correlation of CAF and Creatinine/eGFR in Renal Transplant Patients

The cWP of CAF and creatinine was $r = 0.68$ ($p < 0.001$; table 2). When we calculated the correlation of logarithmic concentrations, cWP was even stronger ($r = 0.86$, $p < 0.001$; table 2). The cBP was somewhat weaker, but still $r = 0.55$ ($p < 0.001$; table 2) for values on the original scale and $r = 0.74$ ($p < 0.001$; table 2) for logarithmic data. When we compared logarithmic CAF and eGFR, cWP was $r = -0.86$ ($p < 0.001$; table 2) and cBP was $r = -0.77$ ($p < 0.001$; table 2). We did not calculate the correlation of raw values, since the logarithm of eGFR and the logarithm of creatinine values follow a linear relationship.



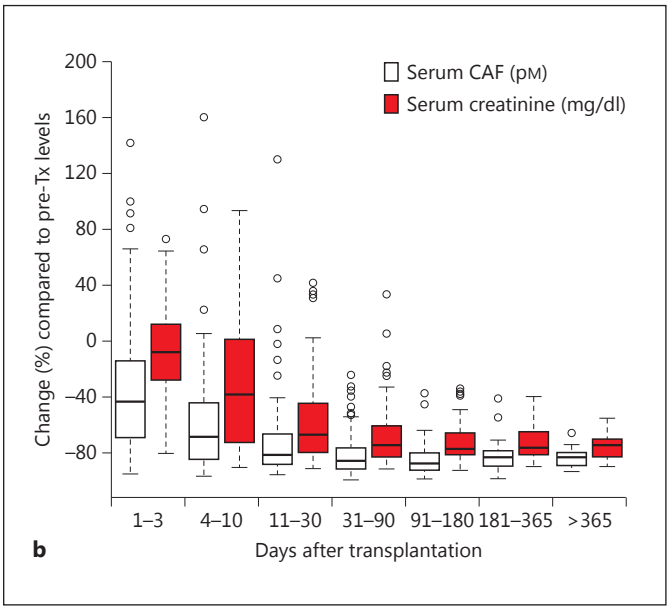


Fig. 1. a Development of median serum CAF and creatinine levels in renal transplant recipients before and after transplantation (mean values). **b** Change of serum CAF/creatinine levels at each time period in reference to pre-Tx levels. Results are presented as boxplots: the horizontal black line indicates the median, the lower and the upper limit of the box are the 25th and 75th percentile. The length of the box corresponds to the interquartile range (IQR). Within the box there are the central 50% of all samples. The whiskers represent the lowest datum still within 1.5 IQR of the lower end of the box, and the highest datum still within 1.5 IQR of the upper end of the box. Any data not included between the whiskers, plotted with circles, indicate extreme values.

Table 2. Serum CAF levels, serum creatinine levels and eGFR (MDRD) in renal transplant recipients before and after transplantation and in healthy volunteers

Samples	Serum CAF, pM	Serum creatinine, mg/dl	CAF–creatinine		eGFR (MDRD), ml/min	CAF–eGFR	
			cWP	cBP		cWP	cBP
Pre-Tx	1,115.0 (258.4–3,990.0)	6.85 (3.1–15.7)			8 (4–19)		
POD 1–3	562.8 (101.6–2,113.0)	6.4 (1.7–12.7)	untransformed	untransformed	9 (5–42)		
POD 4–10	293.8 (67.8–1,933.0)	3.9 (0.8–17.6)	values	values	17 (4–99)		
POD 11–30	178.7 (46.2–1,337.0)	2.1 (0.8–10.0)	0.68*	0.55*	33 (6–82)	logarithmic	logarithmic
POD 31–90	145.1 (6.7–851.0)	1.6 (0.7–8.0)			43 (8–130)	transformation	transformation
POD 91–180	141.4 (13.7–424.9)	1.7 (0.8–5.0)	logarithmic	logarithmic	43 (12–120)	–0.86*	–0.77*
POD 181–365	146.9 (11.3–483.1)	1.6 (0.7–3.7)	transformation	transformation	43 (14–129)		
>365 POD	143.6 (46.3–287.6)	1.7 (1.0–2.4)	0.86*	0.74*	44 (23–78)		
Healthy volunteers	56.6 (20.0–109.5)	0.8 (0.5–1.1)			93 (59–155)		

Data are presented as median (min–max). * $p < 0.001$.

Development of CAF and Creatinine Concentrations/eGFR in Kidney Transplant Recipients

The median pre-Tx CAF concentrations were 19-fold higher in kidney transplant patients before transplantation compared to healthy volunteers (1,115.0 (258.4–3,990.0) vs. 56.0 (20.0–109.5) pM). The median serum creatinine concentrations in healthy subjects were 0.78 (0.53–1.08) mg/dl. During POD 1–3 there was a 44% decrease of relative values ((POD 1–3 – pre-Tx)/pre-Tx) in CAF concentrations (562.8 (101.6–2,113.0) pM, $p < 0.001$; table 2; fig. 1). 1–3 months after transplantation, CAF concentrations were around 86% lower than pre-Tx concentrations (145.1 (6.7–851.0) pM, $p < 0.001$; table 2; fig. 1) and 2.6-fold higher than in healthy volunteers. The

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median creatinine concentrations ((POD 1–3 – pre-Tx)/pre-Tx) relatively decreased only by around 8% from 6.9 (3.1–15.7) mg/dl pre-Tx to 6.4 (1.7–12.7) mg/dl during POD 1–3 (p = 0.001; table 2; fig. 1). 1–3 months after transplantation, creatinine concentrations were 74% lower compared to pre-Tx concentrations (1.6 (0.7–8.0 mg/dl, p < 0.001; table 2; fig. 1) and 2.1 times higher than in healthy subjects.

Comparison of the Development of Postoperative CAF and Creatinine Concentrations within Patients

Comparing the time course of both parameters in the individual patient, CAF concentrations decreased significantly faster from pre-Tx to POD 1–3 than creatinine concentrations (44 vs. 8%, p < 0.001; fig. 1a). From POD 1–3 to POD 4–10 and onwards the decrease of median CAF and creatinine concentrations was not statistically different anymore. However, the percent change of CAF concentrations (e.g. (POD 1–3 – pre-Tx)/pre-Tx) was significantly lower compared to creatinine concentrations at each time period after transplantation with respect to pre-Tx concentrations (p < 0.01 for each time period; fig. 1b).

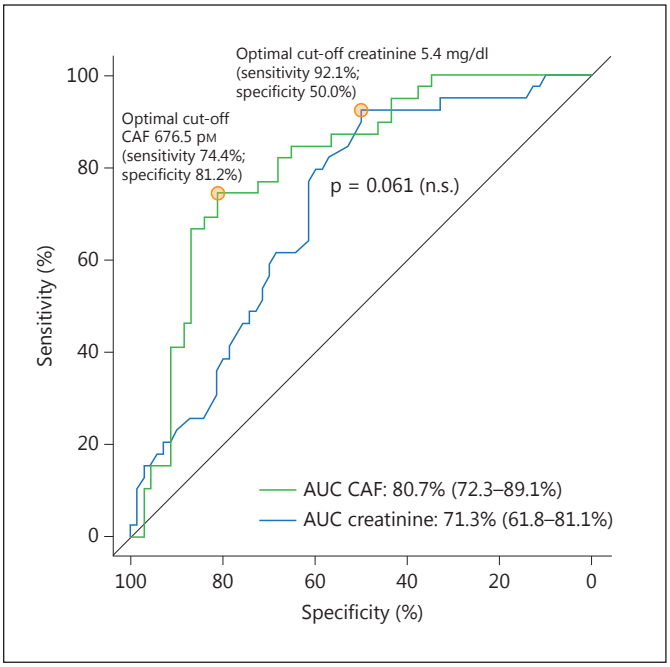

Fig. 2. ROC analysis comparing serum CAF and serum creatinine on POD 1–3 in predicting DGF.

CAF Concentrations and DGF

40 (36.4%) patients experienced DGF as described in the Patients and Methods section. The indication for extracorporeal treatment was poor renal function in 10 patients, hypervolemia in 1 patient, both poor renal function and hypervolemia in 20, and poor renal function, hypervolemia and hyperkalemia in 9 patients. Isolated hyperkalemia was therefore never the only reason for hemodialysis treatment. When we compared the correlation of pre-Tx CAF and creatinine concentrations with DGF, there was no statistically significant difference between patients with DGF and patients with immediate graft function (IGF) (1,137 (366–2,133) vs. 1,031 (258–3,990) pM, p = 0.688). 21 patients in the DGF group lacked residual urinary output; the median CAF concentration in this group of patients was neither statistically different from that of patients with DGF and preserved urinary output (i.e. urinary output >200 ml/day) nor from that of patients with IGF (1,152 vs. 1,009 pM, p = 0.613, and vs. 1,031 pM, p = 0.628). Concerning creatinine concentrations, those were 6.8 (3.7–15.7) vs. 6.9 (3.1–14.2) mg/dl (p = 0.828). During POD 1–3, CAF and creatinine concentrations were significantly higher in the DGF group than in the IGF group (911 (271.7–1,763) vs. 364.6 (102–2,113) pM and 7.1 (2.9–12.7) vs. 5.6 (1.7–12.5) mg/dl, p < 0.001 each). In the DGF group, the median CAF concentrations decreased around 19.9%, whereas the median creatinine concentrations increased by around 4.4%. In the IGF group, the median CAF concentrations decreased about 64.6%, whereas the median creatinine concentrations decreased only around 18.8%. When we created subgroups in the DGF collective, dividing patients who received extracorporeal treatment during the first 3 days versus days 4–7, we saw that also in the latter group CAF concentrations decreased by only 26.7% in the first 3 days (compared to 8.4% in the group with extracorporeal treatment during days 1–3, p = 0.226). This means that also these patients could be distinguished from IGF patients during the first 3 days by using serum CAF concentrations (p < 0.001). In ROC analysis, CAF concentrations during POD 1–3 were moderately accurate in the early detection of DGF concerning sensitivity and specificity with an area under the curve (AUC) of 80.7% (72.3–89.1%; fig. 2). Although statistical significance was just failed (p = 0.061), it was overall superior to creatinine with an AUC of 71.3% (61.8–81.1%) (fig. 2). The optimal cut-off value, which maximizes the sum of sensitivity and specificity for CAF in detecting DGF, was 676.5 pM, resulting in a sensitivity of 74.4% and a specificity of 81.2% (fig. 2). The optimal cut-off for creatinine was 5.4 mg/dl, resulting in a sensitivity of 92.3%, but a specificity of only 50.0% (fig. 2).

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Effect of Different Parameters on CAF and Creatinine Concentrations

By univariate analysis, pre-Tx CAF concentrations in females were significantly higher than in males (1,206 (112.0–3,990.0) vs. 983 (259.0–2,987.2) pM, p = 0.032). Age (ρ = 0.002, p = 0.985), weight (ρ = –0.060, p = 0.547), BMI (ρ = –0.036, p = 0.730), TLC (ρ = 0.022, p = 0.824), CRP (ρ = 0.064, p = 0.516), hemoglobin (ρ = 0.016, p = 0.874), sodium concentrations (ρ = 0.032, p = 0.745), total protein count (ρ = 0.182, p = 0.087), liver damage parameters (GPT ρ = 0.016, p = 0.868; GGT ρ = 0.073, p = 0.461) and underlying disease (χ^2 = 2.681, p = 0.681) showed no correlation to CAF concentrations. To further investigate the question if CAF is released by kidney injury, we compared pre-Tx and posttransplant CAF concentrations in 7 bilaterally nephrectomized patients with 103 non-nephrectomized patients. CAF concentrations tended to be lower in nephrectomized patients without statistical significance (806 (335.1–3,329.6) vs. 1,115 (258.4–3,990.0) pM, p = 0.866).

Discussion

This is the first study demonstrating that circulating blood CAF could serve as a new biomarker for evaluating and monitoring kidney function. Firstly, we could show that CAF concentrations are highly correlated with eGFR as well as creatinine concentrations. CAF concentrations were 19-fold higher in end-stage renal disease patients before transplantation compared to healthy subjects. Secondly, CAF concentrations decreased significantly over a period of 4 weeks to reach stable concentrations at 1–3 months after transplantation. The initial decrease was significantly greater and more rapid than that of creatinine. Thirdly, stable CAF concentrations were significantly lower than creatinine concentrations compared to pre-Tx concentrations, indicating a wider range of amplitude and therefore higher sensitivity for small changes of kidney function. Fourthly, early postoperative CAF concentrations were significantly associated with DGF and detected DGF with good sensitivity and specificity exceeding the overall diagnostic ability of creatinine.

The major finding of our study is that serum CAF concentrations are highly correlated to eGFR/creatinine concentrations and therefore kidney function. There are at least two possible explanations for this finding: circulating CAF concentrations might be mainly produced by extrarenal tissue and elevated CAF concentrations could be generated due to reduced glomerular filtration and clearance. This filtration sensitivity would be analogous to creatinine or cystatin C and could explain the similar kinetics both CAF and creatinine show with improving kidney function after transplantation. Alternately, the cleavage of agrin directly in renal tissue could lead to increased CAF concentrations and a degradation of the glomerular basement membrane and therefore of the glomerulus itself, causing a decline in glomerular function and/or glomerular loss, respectively. However, mice lacking agrin have been generated and show no morphological anomalies in their kidneys and normal kidney function including glomerular function [15, 16]. Additionally, our analysis with nephrectomized patients did not show significant differences compared to patients with kidneys in situ. Thus, reduced renal clearance of CAF is the most likely mechanism for elevated CAF concentrations in chronic kidney disease.

The second finding of our study was the observation of a decrease in CAF blood concentrations with improving graft function after transplantation. There was no specific difference in the development of CAF and creatinine levels concerning donor organ type (i.e. living vs. postmortal and standard vs. extended criteria donor organs). A strong correlation of CAF with creatinine and eGFR for both the cWP and the cBP was detected. In this context the correlation of changes in both parameters exceeded the absolute values correlation, indicating a strong dependence of CAF on renal function. Since absolute creatinine values show high intraindividual differences, the cBP might lack sensitivity when comparing values on the original scale. Interestingly, the logarithmic correlation between CAF and creatinine as well as eGFR was higher than comparing absolute values on the original scale. For eGFR the correlation was also observed for other biomarkers such as cystatin C, NGAL and creatinine [17, 18], but correlation of CAF and GFR in the setting of transplantation was even stronger than previously reported for cystatin C and NGAL [19, 20, 22]. Compared to pre-Tx concentrations, CAF concentrations were significantly lower during each time period after transplantation compared to creatinine. Overall, the range of CAF concentrations in our study was from 145.1 pM (median) at stable graft function 1–3 months posttransplant to 1,115 pM (median) pre-Tx, indicating an induction of nearly 770%, whereas the range of creatinine was only half as much at around 430%. This suggests that CAF may be a more sensitive marker for the detection of even smaller changes of renal function. This observation is strengthened by the finding that serum CAF concentrations decreased significantly faster than creatinine in the early postoperative

phase, whereas creatinine showed a more gradual decline on improvement of transplant function. Similar observations have been published for cystatin C in the setting of kidney transplantation [17, 21] being superior to creatinine in detecting changes in kidney function. However, other studies failed to show superiority of cystatin C as well as NGAL over creatinine in reflecting renal function in different settings [22–26]. Additionally, cystatin C concentrations were observed to rise again in the postoperative period after renal transplantation independently from renal function [21, 27], therefore limiting its use under these clinical circumstances.

The third major finding is that CAF concentrations during POD 1–3 detected DGF, although statistical significance was just failed, with higher overall specificity and sensitivity than creatinine. We hold the opinion that the lack of significance is primarily due to the limited number of patients, since only 36.4% (n = 40) of patients experienced DGF. In this group, CAF concentrations on POD 1–3 were 250% higher than in the IGF group, whereas creatinine concentrations only differed by around 27%. Conversely, CAF concentrations decreased by around 65% in the IGF group, compared to 19% in the DGF group. Concerning hemodialysis treatment in the DGF group, the question arises if CAF is removed from serum with conventional high-efficient, high-flux hemodialysis treatment and therefore falsifies serum levels. In another cohort of chronic hemodialysis patients, we could show that CAF is not removed from serum with that kind of treatment [unpubl. data] and so serum levels in the DGF group are unaltered. In ROC analysis, CAF concentrations during POD 1–3 detected DGF moderately accurate with an AUC of 80.7%, exceeding the level of creatinine by nearly 10%. Concerning sensitivity and specificity, the optimal cut-off value was 677 pM, resulting in a sensitivity of 74.4% and a specificity of 81.2%. It was superior to creatinine on nearly every point on the ROC analysis curve. Urine NGAL and IL-18 have been positively evaluated for the same purpose [28–30], but urine is often hard to assess. Whereas serum IL-18 failed to be of great value for the prediction of DGF in renal transplant patients [31, 32], results on NGAL are conflicting [32–35]. Blood cystatin C concentrations were beneficial in some studies [32, 36, 37], but failed in others to correlate accurately with allograft function in the early postoperative phase [21, 27, 38]. However, compared to these biomarkers, CAF appears to be a promising marker in this setting.

Concerning parameters possibly influencing CAF concentrations, we found that in univariate analysis females had slightly higher CAF concentrations. Apart from the influence of gender, which is also known for creatinine and cystatin C [39], no other physical condition had a major impact on serum CAF concentrations. CAF seems to be a robust parameter for kidney function independent from individual parameters.

The interpretation of the current study is limited by the fact that our data are observational data and retrospectively analyzed. Due to the retrospective analysis of data, we did not compare CAF with the current gold standard inulin clearance. We could not assess CAF concentrations in the urine since patients often lack urine before transplantation. Additionally, the CAF and creatinine concentrations were assessed during time periods but not on specific postoperative days.

Taken together, CAF might be a potential new and rapid biomarker for kidney function with a high level of sensitivity and specificity that could possibly exceed the use of creatinine and cystatin C. Importantly, CAF might serve as a good clinical biomarker to detect DGF accurately in kidney transplant recipients. Based on the results of our study, future eventually prospective clinical trials evaluating CAF in different clinical settings such as acute kidney failure or chronic diseases should be initiated.

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Disclosure Statement

Stefan Hettwer and Pius Dahinden are currently employed by Neurotune AG, Schlieren, Switzerland. The remaining authors of this article have no conflicts of interest to disclose.

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